Effects of Obesity and Caloric Intake on Biliary Lipid Metabolism in Man

LYNN J. BENNION and SCOTT M. GRUNDY

From the Phoenix Clinical Research Section, National Institute of Arthritis, Metabolism, and Digestive Diseases, Phoenix, Arizona 85016, and Department of Medicine, University of California at San Diego, Veterans Administration Hospital, La Jolla, California 92161

ABSTRACT The effects of obesity and caloric intake on biliary lipid metabolism were investigated in a series of related studies. The degree of saturation of gallbladder bile with cholesterol was found to be significantly higher in a group of 23 obese healthy subjects than in a group of 23 nonobese controls matched for age, sex, and race. Bile was also significantly more saturated in 11 obese subjects before than after weight reduction. To determine whether supersaturated bile in obesity is due to excessive secretion of cholesterol or to deficient secretion of bile acids and phospholipids, the hepatic outputs of these three lipids were measured during constant duodenal infusion of formula in the same 11 subjects before and after weight reduction. Weight reduction resulted in significant reduction of cholesterol output but not of bile acid or phospholipid output. Moreover, very obese subjects were found to have cholesterol secretion rates markedly higher than less obese subjects previously studied by the same method. In obese subjects, bile was supersaturated with cholesterol despite increased bile acid pool sizes and increased secretion rates of bile acids and phospholipids. Supersaturated bile in the obese could therefore be attributed to a single defect in lipid secretion, namely, an excessive output of cholesterol.

To determine whether the rate of caloric intake can account for the effects of obesity on biliary lipid composition and secretion, nine obese white men were studied on a weight maintenance diet and then during weight reduction on a 1,000 cal diet. As compared to weight maintenance, chronic caloric restriction resulted in reduced outputs of cholesterol, bile acids, and phospholipids, reduced bile acid pool size, and reduced synthesis and fecal excretion of cholesterol. Saturation of bile with cholesterol did not decrease during weight reduction, evidently because of the mobilization of cholesterol from adipose stores and the marked reduction in bile acid and phospholipid output observed during chronic caloric restriction. Acute alterations in caloric infusion rates did not fully reproduce the effects of chronic administration of high and low calorie diets. Likewise, chronic intake of hypercaloric diets by nonobese subjects did not reproduce the cholesterol hypersecretion characteristic of the obese. Thus, increased cholesterol secretion in obese subjects could not be fully explained by the amount of calories they ingested to maintain stable weight.

It is concluded that obesity is characterized by excessive hepatic secretion of cholesterol which results in supersaturated bile.

INTRODUCTION

On the basis of clinical observations, obesity has long been suspected of contributing to cholelithiasis (2). Epidemiologic studies relating obesity to gallstones have thus far been inconclusive, but several studies have reported a positive correlation between obesity and gallstone prevalence (3–10). The causes of this correlation have not been elucidated, but most gallstones in obese patients are composed predominantly of cholesterol (10).

A biochemical approach to the etiology of cholesterol cholelithiasis has been opened by recent advances in understanding of the physicochemical bases of gallstone formation (11). Cholesterol, insoluble in water, is solubilized in bile by mixed micelles of bile acids and phospholipids (12–14). Cholesterol precipitation and gallstone formation are favored when bile contains more cholesterol than can be held in stable solution by available bile acids and phospholipid (11, 15–19). It is generally agreed that supersaturation of bile occurs when cholesterol is in the range of 6–10 molar percent of the

This work was presented at the American Federation for Clinical Research, 5 May 1974, Atlantic City, N. J. A preliminary report of this work has been published in abstract form (1).

Received for publication 13 November 1974 and in revised form 11 June 1975.

total lipid mixture of cholesterol, bile acids, and phospholipids (11, 20, 21).

The relative proportion of cholesterol in bile will obviously be increased by whatever factors induce a disproportionate decrease in hepatic secretion of bile acids or phospholipids or an increase in cholesterol secretion. The importance of a deficiency of solubilizing lipids (bile acids and phospholipids) in the production of supersaturated bile has been shown by several studies (22–26). On the other hand, studies in our laboratory have demonstrated that an absolute increase in secretion of cholesterol also contributes to formation of supersaturated bile in both American Indian and young white women with cholesterol gallstones (18, 19). In both Indian and white women, however, gallstone patients were more obese than control subjects, suggesting that obesity may have been responsible for the enhanced cholesterol secretion. Previous studies have established that obesity is associated with an increased production of cholesterol (27-32), and since the biliary tree is the main route of cholesterol excretion in man, it might be expected that biliary secretion of cholesterol would be increased in obesity.

The present work was undertaken to study the relation between obesity and biliary lipids; specifically, it was designed to answer the following questions: (*a*) Is obesity associated with increased saturation of bile with cholesterol? (*b*) If so, is this increased saturation due to excessive cholesterol secretion or deficient secretion of bile acids and phospholipids? (*c*) Will weight reduction reverse any increased saturation associated with obesity? (*d*) Are the effects of obesity on bile mediated by the level of caloric intake required to maintain the obese state?

METHODS

Several related studies were carried out in the Phoenix Clinical Research Section (National Institute of Arthritis, Metabolism, and Digestive Diseases; National Institutes of Health), Phoenix, Ariz. and in the Special Diagnostic and Treatment Unit, Veterans Administration Hospital, San Diego, Calif. The experimental design and location of these studies are outlined in Table I, and a detailed description of each study is given below. All subjects were in good health and were without hepatobiliary or metabolic disorders except as explicitly noted below. Urinalysis, serum glutamic oxaloacetic transaminase (GOT), alkaline phosphatase, bilirubin, albumen, prothrombin time, thyroxine, fasting blood sugar, urea nitrogen, and creatinine were normal in all subjects. Informed written consent was obtained from each subject before the study.

Experimental design

I. EFFECTS OF OBESITY: STUDIES DURING MAINTENANCE OF STABLE WEIGHT

A. *Composition of lipids in gallbladder bile* was determined in 23 pairs of subjects who were matched according to age, sex, and race. One member of each pair was obese and the other was near normal weight (33). They consisted of five pairs of Indian women, five pairs of Indian men, five pairs of white women, and eight pairs of white men. All were in good health except that the nonobese white men had chronic peptic ulcer disease; they were in the hospital as part of a protocol which provided extensive evaluation for possible vagotomy. None had evidence of hepatobiliary disease, and all were in a good state of nutrition.

TABLE I
Experimental Design

I. Effects of obesity: studies during maintenance of stable weight	
A. Bile lipid composition in matched pairs of obese vs. nonobese persons	
Five pairs of Indian women	[Table II]*
Five pairs of Indian men	
Five pairs of white women	
Eight pairs of white men	[Table III]‡
B. Bile lipid secretion in 16 very obese subjects, compared to previously published studies using same method.	[Table IV]‡
C. Bile lipid composition and secretion in 11 obese subjects	
1. Before weight reduction (Period C-1)	[Tables V, VI]*
2. After weight reduction (Period C-2)	
II. Effects of caloric intake: studies during weight maintenance (eucaloric diet), weight loss (hypocaloric diet), and weight gain (hypercaloric diet)	
A. Bile lipid composition and secretion, bile acid pool size, and steroid synthesis and excretion in obese white men	
1. Eucaloric (Period A-1)	[Tables VII–IX]‡
2. Hypocaloric (Period A-2)	
B. Bile lipid secretion in nonobese subjects	
1. Eucaloric (Period B-1)	[Table X]‡
2. Hypercaloric (Period B-2)	
3. Hypocaloric (Period B-3)	

* Studies performed at Phoenix Clinical Research Section.
‡ Studies performed at Veterans Administration Hospital Special Diagnostic and Treatment Unit.

B. *Hepatic secretion rates of biliary lipids* were determined during weight maintenance before caloric restriction in 16 very obese subjects. These comprised all subjects weighing more than 150% of ideal weight (33) included in studies I-C and II-A below. Seven of these subjects were studied in Phoenix (study I-C before weight loss) and nine in San Diego (study II-A before caloric restriction). Their biliary lipid secretion rates were compared with those of other groups of subjects previously studied by the same method in our laboratories (Table IV). These latter groups include American Indian women with cholesterol gallstones, non-Indian women with gallstones, Indian men without gallstones, and white women without stones; the results of these patients have been presented previously (18, 19). For additional comparison, data from 10 nonobese white men are also included; these men were recently studied in our laboratory as part of an investigation of lipid metabolism in mild fasting hypertriglyceridemia. They were in good health and without evidence of hepatobiliary disease.

C. *Composition and secretion of biliary lipids* were studied during weight maintenance before and after weight re-

duction in 11 obese subjects. Their age, race, sex, and weight are shown in Table V. All were in good health and normal by the criteria specified above except that one subject (G. D.) had undergone cholecystectomy for gallstones several years previously. During the period of weight maintenance, both before and after weight reduction, four of the subjects were inpatients on the metabolic ward and they consumed a repetitive, solid-food diet containing 40% fat and average daily cholesterol content of 1,230 mg before weight reduction and 1,170 mg after weight reduction. The other seven subjects were outpatients who adjusted their own food intake to maintain constant weight; these subjects were employees at or near the hospital, and their weights were measured daily at the metabolic ward. Before weight reduction, while in the weight-maintaining state, lipid composition of gallbladder bile and the hepatic secretion rates of biliary lipids were determined. After completion of these initial studies, the subjects lost weight by adherence to a solid food diet containing 300–500 cal per day. This diet was provided daily at the metabolic ward with daily vitamin supplementation and liberal fluid intake. Serum potassium, uric acid, and creatinine were monitored weekly. Intermittent oral KCl supplementation was required in two subjects to maintain serum potassium within the normal range of 3.5–5.0 meq/liter. After weight loss, constant weight was re-established and maintained for an average of 23 days (range 11–44 days); this period was allowed to eliminate effects of the catabolic state. The studies of biliary lipid composition and secretion listed above were then repeated in each subject.

II. Effects of caloric intake: studies during weight maintenance (eucaloric diet), weight loss (hypocaloric diet), and weight gain (hypercaloric diet)

A. *Bile lipid composition and secretion, steroid synthesis and excretion, and bile acid pool size* in 10 obese subjects were determined during weight maintenance on a eucaloric diet and then during weight loss on a hypocaloric diet. All 10 subjects were hospitalized on the metabolic ward for 3–5 mo. After a brief period of stabilization in the hospital, they were started on a eucaloric diet and maintained at constant weight for approximately 1 mo (Period A-1). Throughout this period, all stools were collected for measurement of cholesterol balance. The caloric content of the diet was then reduced to 1,000 cal per day, and stool collections were discontinued for 1–2 mo. Then, while the subjects were still on the hypocaloric diet, cholesterol balance studies were resumed and continued for several weeks (Period A-2). Throughout both the eucaloric and the hypocaloric periods, the subjects consumed a diet of mixed solid food and formula containing 40% of calories as fat. The diet consisted of two solid food and three formula feedings per day; calories were distributed equally among feedings. Formulas contained milk protein (RI-5, Ross Laboratories, Columbus, Ohio), dextrose, and lard. Solid foods consisted of chicken stripped of fat, nonfat bread, dry cereal, potatoes, sugar, and lard. The composition and timing of meals was the same during the eucaloric and the hypocaloric periods, except that during the eucaloric period daily intake of calories ranged from 3,400 to 4,500 and of cholesterol from 188 to 518 mg, and during the hypocaloric period the daily intake of calories was 1,000 and of cholesterol was 61–98 mg. Vitamin and mineral supplements were given daily.

Throughout Periods A-1 and A-2 several samples of fasting gallbladder bile were obtained for lipid analysis, and at the end of Periods A-1 and A-2 biliary lipid secretion rates and bile acid pool size were measured. In several subjects, adipose tissue biopsies were taken during Periods A-1 and A-2 to determine the ratio of cholesterol to triglycerides and to permit estimation of the extent of mobilization of adipose tissue cholesterol during weight reduction.

B. *Biliary lipid secretion rates and bile acid pool size* in two nonobese subjects were determined during weight maintenance on a eucaloric diet (Period B-1), again on a hypocaloric diet (Period B-2), and then on a hypercaloric diet (Period B-3). The level of caloric intake of these nonobese subjects during Periods B-2 and B-3 was the same as that of the obese subjects(above) during Periods A-1 and A-2, respectively. The purpose of this study was to see whether the level of caloric intake can alone account for the changes in biliary lipid metabolism observed in obesity or whether differences between obese and nonobese persist even during identical dietary intake. Dietary composition was similar throughout this study to that described above. Each period was approximately 1 mo, and measurements of biliary lipid outputs and bile acid pool size were made at the end of each period.

Techniques of sample collection and analysis

Gallbladder bile was collected after an overnight fast through a tube positioned in the second portion of the duodenum. After stimulation of gallbladder contraction, 40–80 ml of bile was collected on ice by siphonage through the tube, mixed well, and a 3–10-ml portion taken for analysis. Samples were analyzed for cholesterol, bile acids, and phospholipids, as described previously (34).

Composition and secretion rates of biliary lipids during formula infusion were determined by the method of Grundy the Metzger (34) as follows. The night before the study the subjects swallowed a 3-lumen tube. The next morning the tube was positioned with X-ray guidance in the duodenum so that two proximal outlets were adjacent to the ampulla of Vater and the third outlet was 10–12 cm distal. After collection of gallbladder bile, as above, that portion of the gallbladder bile which was not saved for analysis was returned to the subject via the tube. Then a liquid formula containing 40% of calories as lard was infused continuously through one of the proximal outlets for the remainder of the study. β-sitosterol or [^{14}C]cholesterol (New England Nuclear, Boston, Mass.) was infused with the formula as a dilution marker. After allowing 4 h for gallbladder contraction and for stabilization of hepatic bile secretion, hourly samples were obtained from the second proximal and distal outlets by slow continuous aspiration. Lipid composition of stimulated hepatic bile was determined by analysis of the samples obtained at the proximal aspiration port. The rate of cholesterol output was determined from its ratio to the dilution marker at the distal port. Outputs of bile acids and phospholipids were then calculated from their concentration ratios to cholesterol at the proximal outlet. Evidence that the gallbladder is relatively inactive after 4 h of infusion, so that outputs of lipid at the ampulla of Vater should closely approximate their hepatic secretion rates, has been previously published (34).

In study I-C, the rate of formula infusion was the same before and after weight reduction; in studies II-A and II-B formula was infused at an hourly rate of one-twenty-fourth of the daily caloric intake of the current dietary period. After measurements of lipid secretion rates had been made in study II-A, the rate of formula infusion was

altered to examine the effects of acute changes in caloric intake on biliary lipid secretion.

Individual bile acids. The relative proportions of cholic, chenodeoxycholic, and deoxycholic acids were determined by gas-liquid chromatography (GLC) of the trimethylsilyl (TMS)[1] ethers on a 1% Hi-Eff 8BP column (Applied Science Laboratories Inc., State College, Pa.) as described previously (35). Comparison to a standard mixture of bile acids injected with each GLC run permitted correction for differential detector responses of these three bile acids. In isolation of bile acids for methylation, deconjugation was effected using the enzyme cholylglycine hydrolase (Schwarz-Mann, Orangeburg, N. Y.).

Bile acid pool size was estimated at the time of biliary lipid studies by a method recently described (36). In brief, 5 μCi of [24-^{14}C]cholic acid (New England Nuclear) in 10 ml of ethanol were flushed with water through the distal lumen of the duodenal tube at the outset of formula infusion. After allowing 4 h for equilibration, the ratio of isotope to total bile acids ("specific activity") became constant (36). A mean specific activity was determined on hourly samples over the next 6 h, and the total pool of bile acids was determined by dividing the dose of radioactivity given by the mean specific activity.

Cholesterol balance studies were carried out by methods described previously (37–40). Analyses were carried out entirely by chemical procedures. To correct for losses or degradation of neutral steroids during intestinal transit, β-sitosterol was given in small doses (less than 500 mg/day) in capsule form (39), and chromic oxide was given to correct for variations in fecal flow and losses of acidic steroids (40).

Adipose tissue biopsies were obtained by open surgical biopsy from the abdominal wall. Samples were carefully dissected to remove obvious connective tissue. A weighed piece of tissue was transferred to a tissue grinder and homogenized in 10 ml ethanol with a Teflon pestle. The resultant mixture was heated at 65°C until all lipids were dissolved, and the solution was brought to 100 ml with ethanol. Cholesterol was determined on the ethanolic solution by GLC analysis of the TMS ethers using 5α-cholestane as internal standard. Triglycerides were measured on appropriate dilutions by the Auto Analyzer II (Technicon Instruments Corp, Tarrytown, N. Y.) (41).

Data computation

Cholesterol synthesis should be equal to cholesterol balance (i.e., the difference between intake and excretion of cholesterol and its products) in the metabolic steady state. During weight maintenance (period A-1), subjects were presumably in a steady state, and synthesis could be estimated directly from balance measurements. During caloric restriction (period A-2), cholesterol balance alone could not be equal to synthesis because a metabolic steady state did not exist; in this period, cholesterol was being mobilized from adipose tissue and possibly other tissues. Although a precise measurement of mobilized cholesterol cannot be made, a rough estimate should be possible from the rate of weight loss. Table IX is laid out according to our method of calculation. The assumption was made that after several weeks of weight reduction, the daily rate of weight loss approximates the loss of triglyceride from adipose tissue. We also assumed that during this period, cholesterol and triglycerides were lost proportionately from adipose tissue. The cholesterol: triglyceride ratio in adipose tissue, measured in biopsy specimens as above, multiplied by the rate of triglyceride loss should equal the rate of cholesterol loss from adipose tissue. When the amount of cholesterol lost from adipose tissue and the amount of cholesterol ingested are both subtracted from total fecal steroid excretion, the remainder should approximate endogenous cholesterol synthesis.

Lipid composition of bile is expressed as molar percent cholesterol, bile acids, and phospholipids, according to Admirand and Small (11). The percent saturation of bile with cholesterol was calculated according to the solubility limit described by Hegardt and Dam (20) and Holzbach et al. (21), as well as that of Admirand and Small (11), using the equations suggested by Thomas and Hofmann (42).

Secretion rates of biliary lipids were calculated as previously described (34). To permit comparison of biliary lipid secretion rates between groups of different body size (Table IV), values for lipid outputs were normalized to mg/h per 70 kg of ideal body weight. The method and rationale for this normalization have been previously presented (18, 19).

Statistical significance of data was evaluated by Wilcoxon's matched-pairs signed-ranks test and by Student's *t* test for paired data (43, 44). These methods gave the same result in every instance except where explicitly noted.

RESULTS

I. Effects of obesity: studies during maintenance of stable weight

A. *Lipid composition of gallbladder bile* for the 15 pairs of obese vs. nonobese normal volunteers studied in Phoenix is shown in Table II. In 14 of the 15 matched pairs, the molar percent cholesterol was higher in the obese subject than in the nonobese control. The average molar percent of cholesterol in gallbladder bile of the obese subjects was 9.7, whereas that of the nonobese controls was significantly lower, i.e., 6.5 ($P < 0.01$). The mean molar percent bile acid in the bile of the obese subjects was significantly lower than that of the nonobese matched controls (67.2 vs. 74.0, $P < 0.05$).

Lipid composition of gallbladder bile in the eight pairs of age-matched white men studied in San Diego is given in Table III. The mean molar percent cholesterol in the obese subjects was 11.1 compared with 6.7 for the nonobese controls ($P < 0.05$). In seven of the eight matched pairs, molar percent cholesterol was higher in the bile of the obese subject.

When the data from the two above studies are combined, the molar percent cholesterol in gallbladder bile is significantly higher ($P < 0.01$) and the molar percent bile acid significantly lower ($P < 0.02$) in the obese subjects than in the nonobese controls. Moreover, calculation of the percent saturation of bile with cholesterol, using either the line of equilibrium solubility (20, 21) or that of Admirand and Small (11), again shows the bile of the obese subjects to be significantly more saturated than that of the nonobese

[1] *Abbreviation used in this paper:* TMS, trimethylsilyl.

TABLE II

Lipid Composition of Gallbladder Bile in 15 Pairs of Obese Versus Nonobese Human Volunteers Matched for Age, Sex, and Race

Subject	% ideal weight*	Age	Race/sex	Bile lipid composition		
				Cholesterol	Bile acid	Phospholipid
					molar %	
Obese subjects						
1. D. H.	216	21	Ind/F	13.4	58.3	28.3
2. R. S.	163	19	Ind/F	10.1	65.5	24.4
3. M. F.	177	32	Ind/F	8.7	66.2	25.1
4. V. M.	158	34	Ind/F	11.1	67.4	21.6
5. F. B.	273	36	Ind/F	8.4	78.8	12.8
6. L. M.	277	34	Ind/M	17.1	59.8	23.1
7. R. A.	172	18	Ind/M	7.5	64.6	27.9
8. L. S.	178	43	Ind/M	8.9	66.0	25.1
9. H. M.	225	25	Ind/M	10.3	60.9	28.8
10. B. C.	141	27	Ind/M	5.1	70.2	24.7
11. C. M.	160	46	Cau/F	9.5	79.4	11.1
12. M. M.	137	19	Cau/F	12.1	54.4	33.4
13. M. R.	135	21	Cau/F	8.7	69.7	21.6
14. N. F.	138	25	Cau/F	8.4	70.0	21.6
15. M. P.	125	22	Cau/F	5.9	76.8	17.2
Mean	178	28.1		9.7‡	67.2§	23.1
Nonobese subjects						
1. C. G.	112	23	Ind/F	11.1	73.0	15.9
2. N. E.	103	20	Ind/F	4.4	77.3	18.3
3. F. T.	93	28	Ind/F	7.4	72.3	20.3
4. A. M.	108	31	Ind/F	10.7	67.6	21.6
5. L. H.	108	36	Ind/F	8.3	64.7	27.0
6. R. W.	115	33	Ind/M	5.1	85.8	9.1
7. R. N.	81	22	Ind/M	6.7	75.5	17.8
8. E. S.	107	39	Ind/M	4.1	70.1	25.8
9. R. K.	98	23	Ind/M	6.3	74.7	19.0
10. W. A.	119	29	Ind/M	8.5	67.8	23.7
11. T. M.	103	62	Cau/F	6.8	71.4	21.8
12. E. B.	100	19	Cau/F	3.9	81.4	14.6
13. L. J.	108	21	Cau/F	6.0	76.2	17.8
14. M. T.	104	30	Cau/F	3.8	78.0	18.2
15. C. P.	109	23	Cau/F	4.5	73.4	22.1
Mean	105	29.3		6.5	74.0	19.5

The subject numbers indicate the matched pairs.

* Calculated according to life insurance tables (33).

‡ $P < 0.01$, compared to nonobese subjects.

§ $P < 0.05$, compared to nonobese subjects.

controls ($P < 0.01$). As shown in Fig. 1, this difference was independent of race or sex. Since pairs were matched for age, this difference was also apparently independent of age.

B. *Bile lipid secretion* was measured in 16 very obese subjects to elucidate mechanisms responsible for the highly supersaturated bile found in obesity. The results are summarized in Table IV; secretion rates for the individual subjects are given in Tables VI and VII. Table IV compares lipid secretion rates in two groups of very obese subjects with three groups of relatively nonobese subjects without gallstones (Indian and non-Indian men and non-Indian women) and two groups of moderately obese subjects with gallstones. The results show that increased cholesterol saturation in very obese subjects cannot be explained by a decreased output of

TABLE III

Lipid Composition of Gallbladder Bile in Eight Obese Caucasian Males and Eight Age-Matched Caucasian Male Controls

Subject	% ideal weight‡	Age	Bile lipid composition* Cholesterol	Bile acid	Phospholipid
			molar %		
Obese subjects					
1. S. A.	191	26	12.8	61.6	25.6
2. V. C.	184	37	11.0	59.9	29.1
3. D. L.	225	46	12.7	61.3	26.0
4. R. J.	152	48	14.1	63.0	22.8
5. J. M.	155	50	11.3	57.3	31.3
6. T. P.	153	50	8.5	67.7	23.8
7. L. McG.	165	55	10.0	66.9	23.1
8. W. R.	227	56	8.4	62.7	28.8
Mean	182	46	11.1§	62.6	26.3
Nonobese patients					
1. S. A.	104	24	4.6	69.2	26.1
2. C. S.	119	31	6.5	70.6	22.9
3. G. F.	91	44	2.6	86.1	11.4
4. E. H.	85	49	4.2	79.6	16.3
5. E. E.	94	52	7.5	58.4	34.1
6. B. H.	123	53	8.3	62.9	28.8
7. O. R.	104	55	8.9	70.7	20.4
8. J. O.	119	60	10.7	65.9	23.4
Mean	105	46	6.7	70.4	22.9

The subject numbers indicate the matched pairs.

* The bile lipid composition for the obese subjects represents the results from the single sample that is nearest to the mean of multiple samples shown in Table VII. The mean molar percent for cholesterol was 11.1 for the single samples and 10.9 for the multiple samples in the same patients.

‡ Calculated according to life insurance tables (33).

§ $P < 0.05$, compared to nonobese subjects.

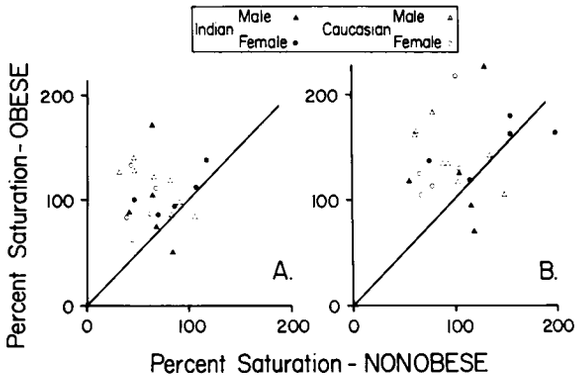

FIGURE 1 Cholesterol saturation of gallbladder bile in 23 pairs of humans matched for age, sex, and race. On the left (A) are shown the data calculated according to the solubility limits of Admirand and Small (11, 42) and on the right (B) according to the equilibrium solubility limit of Hegardt and Dam (20) and Holzbach et al. (21). Each point represents bile compositions from one matched pair: the percent saturation of gallbladder bile from the obese subject is plotted on the ordinate against that of the nonobese matched control on the abscissa. If there were no relationship between obesity and bile saturation, the points would tend to fall about the line of identity at 45°. A significant association of obesity and increased saturation is shown by the fact that most of the points ($P < 0.01$) fall above the line of identity.

solubilizing lipids: bile acid outputs were not reduced and outputs of phospholipids were actually elevated in the obese subjects. On the other hand, cholesterol outputs were markedly increased in these very obese patients.

C. Bile lipid composition and secretion for 11 subjects studied before and after weight reduction are shown in Tables V and VI. The 11 subjects lost an average of 25 kg or 43% of ideal body weight. In every instance, the molar percent cholesterol in gallbladder bile was greater before weight loss than after. Mean molar percent cholesterol before weight loss was 12.0; after weight

TABLE IV

Biliary Lipid Outputs in Two Groups of Very Obese Subjects in Comparison to Several Less Obese Groups

Subjects	Number	Average age	Average percent ideal weight	Cholesterol	Bile acids*	Phospholipids	Bile acid pool size
				mg/70 kg ideal wt/h ±SEM			*mg*
Very obese men studied in San Diego‡	9	45	188	103±9	1,390±90	760±60	4,010±520
Very obese subjects studied in Phoenix‡	7	36	210	103±12	1,100±190	470±80	—
White men without gallstones§	10	49	113	59±4	1,100±120	480±50	3,030±480
Indian men without gallstones‖	7	41	123	51±8	1,370±140	330±40	—
White women without gallstones¶	14	22	99	38±3	1,460±230	350±50	—
Indian women with gallstones‖	17	29	134	67±5	810±60	320±30	—
Non-Indian women with gallstones¶	8	29	147	75±6	1,000±80	350±40	—

* Values for hourly outputs of bile acids are expressed for conjugated bile acids assuming an average mol wt of 500. In a previous study (19), values were expressed in terms of free bile acids.

‡ Original data reported in Tables VI and VIII.

§ Previously unpublished data from subjects normal except for mild hypertriglyceridemia.

‖ Original data reported in reference 19. See footnote*.

¶ Original data reported in reference 18.

TABLE V

Bile Composition in 11 Human Volunteers before and after Weight Reduction

Subject	Age, Sex, Race	Period*	Weight	% ideal weight‡	Gallbladder bile			Stimulated hepatic bile		
					Cholesterol	Bile acids	Phospholipids	Cholesterol	Bile acids	Phospholipids
			kg			*molar %*			*molar %*	
F. B.	35F	C-1	134.5	273	8.4	78.8	12.8	9.3	67.7	23.0
	Ind	C-2	98.2	200	7.0	69.9	23.1	4.8	77.0	18.2
C. J.	22F	C-1	84.5	156	18.6	72.0	9.4	8.3	76.4	15.3
	Ind	C-2	74.0	137	13.8	80.2	6.0	6.4	77.0	16.6
M. H.	47F	C-1	91.3	166	20.7	59.0	20.3	15.2	65.3	19.5
	Ind	C-2	84.0	153	11.3	67.2	21.4	12.8	68.5	18.7
G. D.	26F	C-1	92.0	157	Cholecystectomized			13.2	67.7	19.1
	Ind	C-2	71.5	122				9.4	69.0	21.6
L. M.	34M	C-1	184.3	277	17.1	59.8	23.1	9.2	72.7	18.1
	Ind	C-2	122.0	184	6.9	78.9	14.2	5.6	79.3	15.2
G. H.	45M	C-1	175.0	281	11.5	62.7	25.8	4.9	74.4	20.7
	Ind	C-2	115.0	184	3.9	80.7	15.3	2.8	83.1	14.1
C. M.	46F	C-1	92.2	160	9.5	79.4	11.1	9.4	69.9	20.7
	Cau	C-2	65.9	115	2.8	75.6	21.5	2.5	81.6	16.0
J. E.	19F	C-1	66.4	125	10.6	69.1	20.3	6.6	76.6	16.9
	Cau	C-2	55.0	104	9.0	70.6	20.4	6.0	79.5	14.5
N. F.	25F	C-1	73.3	138	8.4	70.0	21.6	5.6	77.1	17.4
	Cau	C-2	61.9	117	8.2	72.1	19.7	7.3	76.3	16.4
E. B.	19F	C-1	63.7	121	8.7	70.5	20.8	5.1	80.8	14.2
	Cau	C-2	52.6	100	3.4	81.4	14.6	3.8	85.2	11.0
M. T.	30F	C-1	72.3	139	6.2	70.4	23.5	4.1	76.8	19.1
	Cau	C-2	54.2	104	3.8	78.0	18.2	5.1	77.8	17.0
Mean	32 yr	C-1	102.7	181	12.0	69.2	18.9	8.3	73.2	18.5
		C-2	77.7	138	7.1	75.5	17.4	6.0	77.7	16.3
Percent change			−24	−24	−41	9	−7	−29	6	−12
Significance			$P < 0.01$	$P < 0.01$	$P < 0.01$	NS	NS	$P < 0.05$	NS	NS

* Period C-1: before weight reduction. Period C-2: after weight reduction. In both periods, subjects were at weight maintenance on eucaloric diets.

‡ Calculated according to life insurance tables (33).

loss it was 7.1.. This difference was significant at a level of $P < 0.01$. The percent saturation of gallbladder bile with reference to the metastable-labile limit (11) was higher before weight loss than after in every subject studied ($P < 0.01$). When calculated relative to equilibrium solubility (20, 21), the percent saturation was higher before weight loss than after in every subject except one ($P < 0.01$). Stimulated hepatic bile obtained at the ampulla of Vater during formula infusion was also more highly saturated in the more obese state in 8 of the 11 subjects. Before weight loss, the mean molar percent cholesterol in stimulated hepatic bile was 8.3; after weight loss it was 6.0, a decrease significant at a level of $P < 0.05$.

Additional studies were carried out at intermediate weights on five subjects, as shown in Fig. 2. In general, at intermediate weights, intermediate values for bile saturation were found. Four of these subjects were restudied after weight gain. In each instance, they demonstrated on increase in molar percent cholesterol after weight gain. These data indicate that the response of bile composition to obesity is both graded and reversible and that its reversibility is bidirectional.

TABLE VI

Biliary Lipid Secretion in Obese Human Volunteers before and after Weight Reduction

Subject	Age, Sex, Race	Period*	Weight	Cholesterol	Bile acids	Phospholipids
			kg		*mg/h*§	
F. B.	35F	C-1	134.5	91	1,000	520
	Ind	C-2	98.2	25	500	180
C. J.	22F	C-1	84.5	81	970	300
	Ind	C-2	74.0	80	1,270	420
M. H.	47F	C-1	91.3	60	330	150
	Ind	C-2	84.0	74	540	210
G. D.	26F	C-1	92.0	93	620	270
	Ind	C-2	71.5	92	890	430
L. M.	34M	C-1	184.3	146	1,550	600
	Ind	C-2	122.0	76	1,360	400
G. H.	45M	C-1	175.0	72	1,420	620
	Ind	C-2	115.0	44	870	350
C. M.	46F	C-1	92.2	54	520	240
	Cau	C-2	65.9	23	1,010	300
J. E.	19F	C-1	66.4	50	780	270
	Cau	C-2	55.0	49	860	260
N. F.	25F	C-1	73.3	84	1,460	520
	Cau	C-2	61.9	65	900	300
E. B.	19F	C-1	63.7	49	1,030	280
	Cau	C-2	52.6	39	1,140	230
M. T.	30F	C-1	72.3	46	1,100	420
	Cau	C-2	54.2	40	770	270
Mean	32 yr	C-1	102.7	75	980	380
		C-2	77.7	55	920	300
Percent change				−27	−6	−12
Significance				$P < 0.01$‡	NS	NS

* Period C-1: before weight loss. Period C-2: after weight loss, stable weight having been re-established for at least 11 days before study.

‡ When significance is computed by Student *t* test for correlated data, rather than Wilcoxon's matched-pairs signed-ranks test, the decrease in cholesterol secretion is significant only at a level of $P < 0.05$.

§ Each value represents the mean of 8 1-h sample collections.

Lipid secretion studies were performed on these same 11 subjects (study I-C in Table I) before and after weight loss to further clarify the mechanisms responsible for the observed changes in bile composition. As shown in Table VI, cholesterol secretion was higher before weight loss than after in 10 of the 11 subjects. When data from all 11 subjects were averaged, the outputs of all three biliary lipids were greater in the more obese state, but significantly so only for cholesterol. Bile acid and phospholipid secretion showed no consistent or significant change after weight reduction. Thus, the biliary output of cholesterol was disproportionately increased in the obese state. This increase in cholesterol secretion evidently contributed to the formation of bile of increased saturation. There was a significant relationship between the amount of weight lost and the amount of decrease in cholesterol output, with a correlation coefficient (r) of 0.776 ($P < 0.01$). As shown in Fig. 3, those subjects who lost the most weight (F. B., G. H., C. M., L. H.) had the most marked reductions in cholesterol ouput. They were also the subjects who were initially the most obese. In those sub-

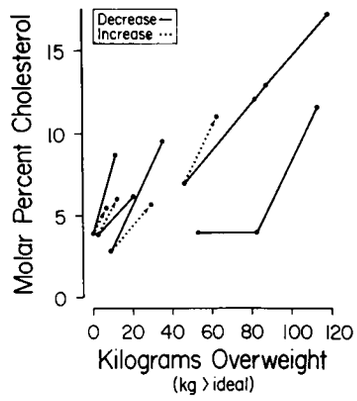

FIGURE 2 Effects of successive changes in weight on gallbladder bile composition. Five subjects (L. M., G. H., M. T., C. M., F. B.) were studied during weight maintenance at three or more different weights. Weight loss resulted in decreased molar percent cholesterol. Weight gain resulted in a return to higher levels of relative cholesterol content. In general, at intermediate weights, gallbladder bile demonstrated intermediate degrees of saturation with cholesterol.

jects whose weights were greater than 50 kg over ideal (F. B., G. H., L. M.), cholesterol output declined an average of 1.2 mg/h per kg of weight lost, whereas subjects whose weights were less than 50 kg over ideal showed an average decline in cholesterol output of only 0.4 mg/h per kg lost.

II. EFFECTS OF CALORIC INTAKE: STUDIES DURING WEIGHT MAINTENANCE, WEIGHT GAIN, AND WEIGHT LOSS

A. *Bile lipid composition* in 10 obese white men on eucaloric and hypocaloric diets is shown in Table VII. Despite the fact that these subjects weighed less while on the hypocaloric diet than on the eucaloric diet, the relative cholesterol content of gallbladder bile was higher in 6 of the 10 while on the hypocaloric diet. Stimulated hepatic bile was generally less saturated than gallbladder bile; it showed no regular or significant decrease in cholesterol saturation during weight loss.

Secretion rates of bile lipids in nine obese men on eucaloric and then hypocaloric diets are given in Table VIII. Outputs of all three types of biliary lipids were significantly lower after the hypocaloric dietary period than the eucaloric period. To test whether the changes observed were due simply to the rate at which formula was being infused, rather than to the chronic level of daily caloric intake, the rate of caloric infusion was varied acutely in several subjects. After measurements of lipid secretion rates had been made during the last 6 h of a 10-h period of hypocaloric infusion, the infusion rate was increased from a rate of 1,000 cal daily to 3,000–4,000 cal. As shown in Table VIII, this return of the infusion rate to approximately that of the eucaloric period did not raise the rate of bile lipid secretion to the levels observed at the end of the eucaloric period. Therefore, the findings of reduced output of cholesterol, bile acids, and phospholipids during chronic caloric restriction cannot be explained by the rate of formula infusion employed, nor can they be reversed by an acute increase in caloric input. Moreover, as shown in Table VIII, a halving of the infusion rate at the end of the eucaloric period does not reduce biliary lipid outputs to the level reached during chronic hypocaloric feeding. Thus, regardless of the rates of caloric infusion used to assess biliary lipid outputs, it is clear that chronic caloric restriction reduces the outputs of cholesterol, bile acids, and phospholipids.

Composition and pool size of bile acids were determined in eight obese white men during formula infusion at the end of the eucaloric and hypocaloric dietary periods. There was no significant difference between the two periods in the relative proportions of cholic, deoxycholic, and chenodeoxycholic acids in stimulated hepatic bile. The size of the total pool of bile acid, however, was significantly greater during weight maintenance on eucaloric diet than during weight reduction on hypocaloric diet. The total bile acid pool during weight maintenance averaged 4,088 mg and during weight reduction averaged 2,378 mg, a significant difference ($P < 0.02$). As noted above, bile acid secretion was also lower during caloric restriction. A reciprocal relationship between pool size and secretion rate of bile acids, previously reported for nonobese gallstone patients (45), was thus not observed in these obese men studied at two different levels of caloric intake.

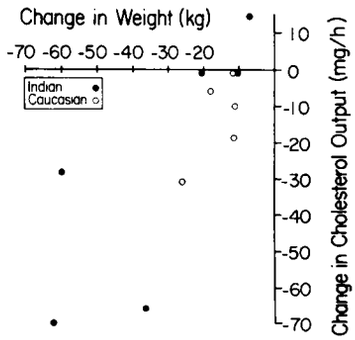

FIGURE 3 Effect of weight reduction on biliary cholesterol output. Hepatic secretion of cholesterol into bile was measured before and after weight reduction in 11 subjects. The amount of weight lost is plotted against the change in cholesterol secretion. The slope of the least squares regression line of weight lost vs. decrease in cholesterol output is significantly different from zero, indicating a significant relationship between these two parameters ($r = 0.776$, $P < 0.01$).

TABLE VII

Bile Lipid Composition during Weight Maintenance and during Weight Reduction in 10 Obese White Males

Subject	Age	Period* (n)	Weight‡	Gallbladder bile			Stimulated hepatic bile		
				Cholesterol	Bile acids	Phospholipids	Cholesterol	Bile acids	Phospholipids
			kg		*molar %*			*molar %*§	
S. A.	26	A-1 (1)	145	12.8	61.6	25.6	6.0	73.3	20.7
		A-2 (3)	127	13.5	62.1	24.5	5.8	74.3	19.9
V. C.	37	A-1 (3)	133	9.7	63.1	27.2	5.5	67.1	27.4
		A-2 (6)	113	9.4	56.5	34.1	3.0	82.2	14.7
R. L.	39	A-1 (4)	124	11.6	62.1	26.3	—	—	—
		A-2 (5)	101	7.4	75.0	17.6	—	—	—
C. L.	41	A-1 (4)	132	9.0	65.2	25.8	5.6	71.8	22.6
		A-2 (3)	117	12.3	56.6	31.1	9.0	67.6	23.4
D. L.	46	A-1 (4)	137	11.9	58.3	29.8	6.5	71.4	22.1
		A-2 (4)	120	16.1	55.1	28.9	2.1	84.8	13.1
R. J.	48	A-1 (3)	124	14.4	58.7	26.9	8.2	65.0	26.8
		A-2 (2)	101	17.1	53.6	29.4	8.5	64.6	26.9
J. M.	50	A-1 (2)	122	9.3	63.6	27.1	6.5	67.7	25.8
		A-2 (7)	96	9.2	66.8	24.0	5.9	69.7	24.3
T. P.	50	A-1 (2)	155	10.0	67.3	22.8	6.8	72.3	20.9
		A-2 (3)	139	12.9	66.5	20.6	7.5	77.1	15.4
L. McG.	55	A-1 (5)	114	10.0	66.9	23.1	5.6	71.5	22.9
		A-2 (7)	99	14.5	52.9	33.1	9.0	61.8	29.3
W. R.	56	A-1 (2)	159	9.7	63.5	26.8	9.0	63.3	27.6
		A-2 (6)	126	8.8	68.1	23.3	7.1	66.0	26.9
Mean	45	A-1 (3)	135	10.8	63.0	26.1	6.6	69.3	24.1
		A-2 (5)	114	12.1	61.3	26.7	6.4	72.0	21.5
Significance				NS	NS	NS	NS	NS	NS

* Period A-1: weight maintenance on eucaloric diet. Period A-2: losing weight on a 1,000 cal diet. Number in parentheses represents number of determinations of gallbladder bile lipid composition during that period.

‡ Weight given for Period A-1 is the stable weight at which the subject was maintained throughout that period. The subject's weight at the end of the period of gradual weight loss on 1,000 cal is given for Period A-2.

§ Values for hepatic bile represent the average for six determinations during the steady-state period of formula infusion.

Cholesterol synthesis and fecal steroid excretion in nine obese men during weight maintenance and reduction are presented in Table IX. Since the diets in both periods contained cholesterol, fecal neutral steroids were derived from both endogenous and exogenous cholesterol. In the eucaloric period (A-1), cholesterol intake was greater, and this may partly account for the greater excretion in that period. During weight loss a similar pattern of change was noted for both neutral and acidic steroids: in some subjects the excretion of acidic and/or neutral steroids was markedly decreased during caloric restriction, while in others it was only slightly decreased; in every case, however, excretion was less during caloric restriction than during weight maintenance. As shown in Table IX, estimated total body synthesis of cholesterol was also less during caloric restriction than during weight maintenance in every subject.

In several subjects, the cholesterol: triglyceride ratio in adipose tissue was higher in Period A-2 than A-1, suggesting that triglyceride may have been mobilized somewhat more rapidly than cholesterol. If so, our estimate of cholesterol mobilization would be correspondingly overestimated in these subjects. Nevertheless, the results provide an interesting comparison between mobilized and newly synthesized cholesterol. They show that mobilization of cholesterol from adipose tissue may

TABLE VIII

Effects of Chronic and Acute Changes in Caloric Intake on Hourly Output of Biliary Lipids and Bile Acid Pool Size in Obese Caucasian Men

Subject	Period*	Weight‡	Dietary calories	Infusion§ calories	Biliary lipid outputs			Bile acid pool size
					Cholesterol	Bile acids	Phospholipids	
		kg	*cal/24 h*	*cal/24 h*		*mg/h*		*mg*
S. A.	A-1	145	3,600	3,600	86	1,340	600	2,390
	A-2	127	1,000	1,000	61	1,010	420	2,760
				4,000	44	830	350	
V. C.	A-1	133	4,000	4,000	86	1,360	870	3,420
	A-2	113	1,000	1,000	32	1,120	310	2,090
				4,000	42	1,490	320	
C. L.	A-1	132	4,000	4,000	87	1,430	700	7,220
				2,000	92	1,180	670	
				1,000	94	1,050	660	
	A-2	117	1,000	1,000	57	560	300	2,100
				3,000	67	670	400	
D. L.	A-1	137	3,800	3,800	92	1,310	630	2,730
				1,600	76	1,040	480	
	A-2	120	1,000	1,000	26	1,370	330	2,300
				4,000	37	960	330	
R. J.	A-1	124	3,600	3,600	124	1,270	820	4,210
				1,800	85	710	520	
	A-2	101	1,000	1,000	69	680	440	2,650
				3,000	59	860	480	
J. M.	A-1	122	3,700	3,700	98	1,320	780	3,110
	A-2	96	1,000	1,000	37	560	310	1,190
				4,000	44	470	340	
T. P.	A-1	155	3,600	3,600	86	1,880	530	4,010
	A-2	139	1,000	1,000	54	720	220	3,400
				4,000	66	950	390	
L. McG.	A-1	114	3,400	3,400	110	1,810	910	5,610
				1,700	96	940	720	
	A-2	99	1,000	1,000	63	560	410	2,630
				3,000	78	900	500	
W. R.	A-1	159	4,500	4,500	157	1,420	970	
	A-2	126	1,000	1,000	83	1,000	630	
				4,000	62	760	230	
Mean	A-1	136	3,800	3,800	103	1,460	760	4,090
	A-2	115	1,000	1,000	54	840	370	2,390
Percent change			−74	−74	−48	−42	−51	−42
Significance					$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$

* Period A-1: subject maintaining weight for a month on eucaloric diet. Period A-2: subject losing weight for more than a month on 1,000 cal diet.

‡ Weight at end of period shown.

§ Rate of constant infusion of liquid formula into duodenum during measurement of biliary lipid outputs. Hourly caloric input was 1/24th of value shown. Formula was infused for 10 h at constant rate. After 4 h for equilibration, six hourly determinations of lipid output were made. Then, as shown by a second value for infusion rate, in several instances the rate of infusion was acutely changed to a new level of caloric intake. Formula was then infused at this new rate for 9 h. After 3 h of equilibration at the new rate, six hourly determinations of lipid output were again made.

‖ Each value represents the mean of six 1-h sample collections.

TABLE IX

Synthesis and Excretion of Steroids during Weight Maintenance and Reduction in Nine Caucasian Men

Subject	Period*	Diet: Cholesterol intake	Fecal excretion: Neutral steroids	Fecal excretion: Acidic steroids	Adipose tissue: Chol/TG	Adipose tissue: TG loss	Adipose tissue: Chol loss	Cholesterol synthesis
		mg/day	*mg/day±SD*		*mg/g*	*g/day*	*mg/day*	*mg/day*
S. A.	A-1	340	1,010±100	470±180	0.94			1,140
	A-2	70	980±150	240±110	0.69	290	270	880
V. C.	A-1	260	1,210±170	490±110	0.76			1,440
	A-2	70	750±60	160±50	2.08	290	220	620
R. L.	A-1	180	950±300	180±60	1.08			950
	A-2	60	570±70	70±10	1.32	350	380	200
D. L.	A-1	210	950±120	610±10	0.91			1,350
	A-2	90	580±100	130±40	1.81	180	160	460
R. J.	A-1	360	1,350±160	300±80	1.24			1,290
	A-2	90	1,220±290	210±50	1.42	390	480	860
J. M.	A-1	290	1,180±340	600±80	0.85			1,490
	A-2	90	520±40	200±40	1.22	300	260	370
T. P.	A-1	360	1,100±120	300±70	1.62			1,040
	A-2	90	860±110	250±70		320	520	500
L. McG.	A-1	240	1,080±170	500±190	1.01			1,340
	A-2	100	940±150	240±110	0.95	200	200	880
W. R.	A-1	520	1,250±180	960±170	1.46			1,690
	A-2	90	840±80	240±50	1.70	380	560	430
Mean	A-1	310	1,120	490	1.10			1,300
	A-2	80	810	190	1.40	300	340	580
Significance			$P < 0.01$	$P < 0.01$				$P < 0.01$

* Period A-1: maintaining weight on eucaloric diet. A-2: losing weight on 1,000 cal diet.

TABLE X

Effects of Caloric Intake on Biliary Lipid Outputs in Two Nonobese Caucasian Men

Patient	Period*	Days	Weight‡	Dietary calories	Infusion calories	Biliary lipid outputs: Cholesterol	Biliary lipid outputs: Bile acids	Biliary lipid outputs: Phospholipids	Bile acid pool size
			kg	*cal/24 h*			*mg/h*		*mg*
D. M.	B-1	27	75.2	2,600	2,600	49±9	1,190±310	480±110	6,570
	B-2	32	78.4	4,000	4,000	66±20	1,560±250	600±150	—
	B-3	29	74.0	1,000	1,000	28±7	630±270	220±70	3,520
W. V.	B-1	30	72.9	3,000	3,000	56±2	1,000±190	480±90	1,870
	B-2	24	76.4	5,000	5,000	46±8	1,160±220	500±90	3,130
	B-3	29	73.8	1,000	1,000	44±3	520±150	190±50	1,220

* Period B-1: subject maintaining stable weight on eucaloric diet. Period B-2: subject gaining weight on hypercaloric diet. Period B-3: subject losing weight on hypocaloric diet. Subjects were studied at end of each period, the durations of which are listed in the adjacent column.

‡ Weight at end of period shown.

§ Each value represents the mean±SD of six hourly determinations which were carried out.

contribute significantly to cholesterol balance during weight reduction. The calculated average for mobilized cholesterol was 338 mg/day. Since cholesterol synthesis was markedly reduced during caloric restriction as compared to weight maintenance (547 vs. 1,301 mg/day), about one-third of the total excretion of endogenous steroids was probably derived from adipose tissue stores. Production of bile acids was also reduced during caloric restriction, as evidenced by decreased bile acid secretion and pool size and decreased acidic steroid excretion, and thus a major fraction of the mobilized cholesterol must have been secreted into bile as cholesterol itself. This excess cholesterol was undoubtedly one factor preventing the reduction of biliary cholesterol saturation during active weight loss.

B. *Bile lipid secretion and bile acid pool size* in two nonobese subjects on eucaloric, hypercaloric, and hypocaloric diets are shown in Table X. The subjects gained about 3.5 kg while on hypercaloric intake (Period B-2) and lost approximately the same amount on hypocaloric intake (Period B-3). Lipid outputs for both subjects during weight maintenance were similar to those previously reported for subjects of normal weight (Table IV), but subject D. M. had an unusually large bile acid pool. As in the obese subjects reported above, caloric restriction resulted in a decreased bile acid pool and decreased output of all three biliary lipids in both subjects. On a hypercaloric diet, however, despite caloric intake equal to that required for weight maintenance in the obese subjects reported above, cholesterol output did not rise to the levels found among obese subjects. Therefore, the high caloric intake (3,500–5,000 cal per day) required to maintain weight in the very obese state cannot alone account for the hypersecretion of cholesterol in that state. This would suggest that something about obesity itself, apart from the caloric intake required to maintain that obesity, contributed to the excessive secretion of cholesterol found in the obese subjects reported above in studies I-C and II-A.

DISCUSSION

The present study shows that obesity is associated with supersaturation of gallbladder bile with cholesterol. Most of our obese subjects had gallbladder bile that was supersaturated by any criteria (11, 20, 21); their bile was clearly more saturated before weight loss than after and more saturated than the bile of matched nonobese controls. Since supersaturated bile evidently precedes and predisposes to gallstone formation (17, 26, 46, 47), it is likely that the clinical association between obesity and gallstones can be explained by the formation of bile containing a relative excess of cholesterol.

Our results indicate that production of supersaturated bile in obese humans is due principally to enhanced secretion of cholesterol into bile. Obese subjects showed increased biliary cholesterol secretion in comparison to previously studied nonobese subjects and in comparison to themselves during and after weight reduction. Previous studies in this laboratory demonstrated that supersaturated bile in American Indian and Caucasian gallstone patients resulted from a dual defect in biliary lipid secretion: excessive cholesterol secretion and diminished bile acid secretion (18, 19). The gallstone patients, however, were more obese than the controls, suggesting that their obesity may have accounted for at least part of these changes. Using the data from those reports, one can calculate that for each kilogram by which the Indian gallstone subjects were heavier than the normal Indian controls, they secreted an average of 1.2 mg more cholesterol per h (19). Similarly, for each kilogram by which the non-Indian gallstone subjects were heavier than their controls they secreted 1.1 mg more cholesterol per h (18). These figures are within the range by which cholesterol secretion decreased in the subjects who lost weight in the present study. Northfield and Hofmann, moreover, found no significant difference in 24-h lipid outputs between seven gallstone patients and seven weight-matched controls (48). It would thus appear that the excessive biliary secretion of cholesterol in the gallstone patients previously studied in our laboratory was due to their obesity.

The present study also demonstrates that decreased outputs of solubilizing lipids are not required for formation of supersaturated bile. Pool sizes of bile acids in our obese men were among the highest reported in the literature (22, 26, 49, 50), and phospholipid outputs were also markedly increased. Thus, our results strongly support the concept that supersaturated bile can occur independently of a decrease in solubilizing lipids and can be due entirely to an increased secretion of cholesterol.

Elevated secretion of cholesterol evidently continues during overnight fasting. This conclusion is implied by our finding that gallbladder bile obtained after overnight fasting was consistently more saturated with cholesterol than was stimulated hepatic bile. Thus, the normal "diurnal" variation in bile lipid composition (48, 51) apparently persists in obesity. Indeed, this variation may be accentuated by obesity.

Since saturation of gallbladder bile in obesity seems to be especially high, as compared to stimulated hepatic bile, it is likely that during overnight fasting even more than during caloric infusion, the secretion of cholesterol is disproportionately increased in obesity. One possible mechanism for such an increase in fasting cholesterol secretion could be that the diurnal variation in cholesterol synthesis, which is related to feeding and fasting (52–54), might be obliterated by obesity. The

obese subject should have ready access to fatty acids as a source of two-carbon fragments for cholesterol synthesis even during fasting. Moreover, in obesity plasma insulin levels are particularly elevated in the fasting state (55), and insulin has been reported to induce increased activity of hydroxymethylglutaryl CoA reductase (56, 57), the rate-limiting enzyme in cholesterol synthesis. If excess cholesterol is produced in the liver during fasting in obese persons, its secretion directly into bile would account for our observation of increased saturation of gallbladder bile in obese subjects.

The increased hepatic secretion of cholesterol in obesity is almost certainly related to excessive production of cholesterol. Several studies have shown that cholesterol synthesis is increased in obesity (27-32), and the nine obese men whose cholesterol synthesis was measured in the present study had higher synthesis rates than those reported for nonobese subjects (reference 30, Table II). The major route for excretion of newly synthesized cholesterol is via the biliary tract, and it might therefore be expected that obese subjects would secrete increased amounts of cholesterol into their bile.

The mechanisms responsible for the increased cholesterol synthesis and secretion in obesity are not known. It is conceivable that the hypertrophied adipose tissue itself might produce increased amounts of cholesterol, but Schreibman et al. (58) did not detect significant amounts of cholesterol synthesis in adipose tissue. On the other hand, some of the excess production of cholesterol might simply be a consequence of the increased caloric intake required to maintain the obese state.

It is possible that greater caloric intake might enhance biliary lipid secretion through a direct affect upon the intestinal tract. For example, Brunner et al. (59) reported that secretion rates of biliary lipids are acutely dependent on caloric intake; these workers found that recycling rates of bile acids in the enterohepatic circulation depend on the level of caloric input, and that secretion rates of other lipids are in turn related to the quantity of bile acids fluxed through the liver. Our results do not support this mechanism as the whole explanation of abnormal lipid secretion in obesity. Although acute increases in the rate of caloric infusion usually resulted in increased lipid secretion rates, these changes were not of the magnitude found after prolonged dietary intake of calories at the levels required to maintain massive obesity.

Another possible cause of the increased secretion of cholesterol in obesity might be the chronic ingestion of a high caloric diet, as required for weight maintenance. The finding that biliary lipids are reduced during caloric restriction supports this possibility. However, the high outputs of cholesterol found in obese subjects could not be reproduced in our two nonobese subjects studied after a month of weight gain on high calorie diet. Thus, increased chronic food intake may enhance biliary lipid secretion by promoting the flux of bile acids in the enterohepatic circulation and by increasing synthesis of all constituents, but obesity per se seems to cause a further, disproportionate increase in cholesterol secretion, especially in the fasting state. The mechanism of the latter effect is not apparent, but it might be related to loss of diurnal variation in cholesterol synthesis.

Since obesity is associated with supersaturated bile, an important question is whether weight reduction will cause a decreased saturation. In these studies, weight reduction has been examined in two phases: (*a*) the transitional period of active weight reduction, and (*b*) the period after weight loss in which a constant weight had been re-established at a new lower level. As discussed below, there are important differences between these two phases in the metabolism of biliary lipids.

An unexpected observation was that saturation of gallbladder bile did not decrease during the period of active weight reduction. Indeed it increased in 6 out of 10 subjects. This same finding has been recently reported in a preliminary communication by Schreibman et al. (60). Increased saturation could be due to further enhancement of cholesterol secretion or to reduction in output of solubilizing lipids. Schreibman and coworkers (60) suggested that mobilization of cholesterol from adipose tissue pools into bile might be responsible for the greater saturation. In absolute terms, however, hepatic secretion of cholesterol in our obese patients was not increased during weight reduction; in fact, there was a significant decline in cholesterol outputs with caloric restriction. Thus, a net increase in saturation during weight reduction must be due to a greater decrease in solubilizing lipids than in cholesterol. Nevertheless, while absolute secretion rates of cholesterol were decreased during the period of weight loss, mobilization of cholesterol from adipose tissue could still play an important role in the greater saturation of this period. Our calculations indicate that a significant fraction of the fecal excretion of cholesterol and its products could have been derived from cholesterol that was lost from adipose tissue. If it were not for this increment of cholesterol, depression of cholesterol secretion during caloric restriction might have been uniform for all lipids so that saturation would not be increased. The contribution of cholesterol from adipose tissue therefore may be significant in preventing a decrease in saturation of fasting gallbladder bile during weight reduction.

After re-establishment of constant weight at a reduced level, gallbladder bile is consistently less saturated with cholesterol than before weight reduction. This decreased saturation apparently results from reduced cho-

lesterol secretion, which in turn is probably secondary to the reduced cholesterol synthesis shown by Miettinen to follow weight loss (30). Maintenance of constant weight at a reduced level differs from active weight reduction in two important respects: (*a*) secretion rates of bile acids and phospholipids are not significantly curtailed, and (*b*) there is no net mobilization of cholesterol from adipose tissue. Both of these differences would favor a decreased saturation of bile during weight maintenance, as compared to the period of caloric restriction.

In conclusion, these studies show that saturation of gallbladder bile is increased in obesity. This increase is primarily the result of enhanced secretion of cholesterol into bile and not of a reduction in solubilizing lipids. During caloric restriction in obese subjects, saturation can actually increase because of decreased output of bile acids and phospholipids, as well as mobilization of cholesterol from adipose stores. When weight maintenance is re-established at a lower level, however, saturation is consistently reduced as compared to the more obese state. Thus, although some subjects may transiently be at increased risk for gallstone formation during active weight reduction, if a lower weight can be established and maintained the chances of gallstone formation would appear to be consistently decreased.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the excellent technical assistance of Mr. Robert Collins, Mr. Elliott Groszek, and Mr. James Hobza; the excellent support rendered by Mrs. Margaret Hendrikx, Miss Liana Povse, Miss Marjorie Whelan, and others of the dietetic and nursing staffs of the Phoenix Clinical Research Section and the Special Diagnostic and Treatment Unit; and the secretarial service provided by Mrs. Harriett Rascon and Mrs. Carolyn Shields. The authors are also grateful for biochemical consultation by Dr. Eunice Flock and for the cooperation of the Indian Health Service.

This work was carried out at the Phoenix Clinical Research Section of the National Institute of Arthritis, Metabolism, and Digestive Diseases and the San Diego Veterans Administration Hospital. The work was supported in part by a research grant from the Veterans Administration and by U. S. Public Health Service grants HL-15556 from the National Heart and Lung Institute and AM-16667 from the National Institute of Arthritis Metabolism and Digestive Diseases.

REFERENCES

1. Bennion, L. J., and S. M. Grundy. 1974. Obesity and lithogenic bile: improvement with weight loss. *Clin. Res.* **22**: 354*a*.
2. Osler, W. 1897. The Principles and Practice of Medicine. D. Appleton, New York. 2nd edition. 466.
3. Mentzer, S. H. 1926. A clinical and pathological study of cholecystitis and cholelithiasis. *Surg. Gynecol. Obst.* **42**: 782–793.
4. Sturdevant, R. A. L., M. L. Pearce, and S. Dayton. 1973. Increased prevalence of cholelithiasis in men ingesting a serum-cholesterol-lowering diet. *N. Engl. J. Med.* **288**: 24–27.
5. Gross, D. M. B. 1929. A statistical study of cholelithiasis. *J. Pathol. Bacteriol.* **32**: 503–526.
6. Wheeler, M., L. L. Hills, and B. Laby. 1970. Cholelithiasis: a clinical dietary survey. *Gut.* **11**: 430–437.
7. Burnett, W. 1971. The epidemiology of gallstones. *Tijdschr. Gastro-Enterol.* **14**: 79–89.
8. Friedman, G. D., W. B. Kannel, and T. R. Dawber. 1966. The epidemiology of gallbladder disease: observations in the Framingham Study. *J. Chronic Dis.* **19**: 273–292.
9. Van der Linden, W. 1961. Some biological traits in female gallstone-disease patients. *Acta Chir. Scand. Suppl.* **269**: 1–94.
10. Marinovic, I., C. Guerra, and G. Larach. 1972. Incidencia de litiasis biliar en material de autopsias y analisis de composicion de los calculos. *Rev. Med. Chile.* **100**: 1320–1327.
11. Admirand, W. H., and D. M. Small. 1968. The physicochemical basis of cholesterol gallstone formation in man. *J. Clin. Invest.* **47**: 1043–1052.
12. Isaksson, B. 1954. On the dissolving power of lecithin and bile salts for cholesterol in human bladder bile. *Acta Soc. Med. Upsal.* **59**: 296–306.
13. Norman, A. 1964. Electrophoretic behavior of bile acids and cholesterol in gall bladder and hepatic bile. *Proc. Soc. Exp. Biol. Med.* **115**: 936–940.
14. Hofmann, A. F. 1965. Clinical implications of physiochemical studies on bile salts. *Gastroenterology.* **48**: 484–494.
15. Small, D. M., and S. Rapo. 1970. Source of abnormal bile in patients with cholesterol gallstones. *N. Engl. J. Med.* **283**: 53–57.
16. Vlahcevic, Z. R., C. C. Bell, Jr., and L. Swell. 1970. Significance of the liver in the production of lithogenic bile in man. *Gastroenterology.* **59**: 62–69.
17. Thistle, J. L., and L. J. Schoenfield. 1971. Lithogenic bile among young Indian women. Lithogenic potential decreased with chenodeoxycholic acid. *N. Engl. J. Med.* **284**: 177–181.
18. Grundy, S. M., W. C. Duane, R. D. Adler, J. M. Aron, and A. L. Metzger. 1974. Biliary lipid outputs in young women with cholesterol gallstones. *Metab. (Clin. Exp.).* **23**: 67–73.
19. Grundy, S. M., A. L. Metzger, and R. D. Adler. 1972. Mechanisms of lithogenic bile formation in American Indian women with cholesterol gallstones. *J. Clin. Invest.* **51**: 3026–3043.
20. Hegardt, F. G., and H. Dam. 1971. The solubility of cholesterol in aqueous solutions of bile salts and lecithin. *Z. Ernaehrungswiss.* **10**: 223–233.
21. Holzbach, R. T., M. Marsh, M. Olszewski, and K. Holan. 1973. Cholesterol solubility in bile. Evidence that supersaturated bile is frequent in healthy man. *J. Clin. Invest.* **52**: 1467–1479.
22. Vlahcevic, Z. R., C. C. Bell, Jr., I. Buhac, J. T. Farrar, and L. Swell. 1970. Diminished bile acid pool size in patients with gallstones. *Gastroenterology.* **59**: 165–173.
23. Swell, L., C. C. Bell, Jr., and Z. R. Vlahcevic. 1971. Relationship of bile acid pool size to biliary lipid excretion and the formation of lithogenic bile in man. *Gastroenterology.* **61**: 716–722.
24. Bell, C. C., Jr., W. C. McCormick, D. H. Gregory, D. H. Law, Z. R. Vlahcevic, and L. Swell. 1972. Rela-

tionship of bile acid pool size to the formation of lithogenous bile in male Indians of the southwest. *Surg. Gynecol. Obst.* **134**: 473–478.
25. Vlahcevic, Z. R., C. C. Bell, Jr., D. H. Gregory, G. Buker, P. Juttijudata, and L. Swell. 1972. Relationship of bile acid pool size to the formation of lithogenic bile in female Indians of the southwest. *Gastroenterology.* **62**: 73–83.
26. Bell, C. C., Jr., Z. R. Vlahcevic, J. Prazich, and L. Swell. 1973. Evidence that a diminished bile acid pool size precedes the formation of cholesterol gallstones in man. *Surg. Gynecol. Obst.* **136**: 961–965.
27. Nestel, P. J., and E. A. Monger. 1967. Turnover of plasma esterified cholesterol in normocholesterolemic and hypercholesterolemic subjects and its relation to body build. *J. Clin. Invest.* **46**: 967–974.
28. Nestel, P. J., H. M. Whyte, and D. S. Goodman. 1969. Distribution and turnover of cholesterol in humans. *J. Clin. Invest.* **48**: 982–991.
29. Miettinen, T. A. 1970. Detection of changes in human cholesterol metabolism. *Ann. Clin. Res.* **2**: 300–320.
30. Miettinen, T. A. 1971. Cholesterol production in obesity. *Circulation.* **44**: 842–850.
31. Nestel, P. J., P. H. Schreibman, and E. H. Ahrens, Jr. 1973. Cholesterol metabolism in human obesity. *J. Clin. Invest.* **52**: 2389–2397.
32. Whyte, H. M., P. J. Nestel, and D. S. Goodman. 1969. Cholesterol distribution and turnover in obesity in man. *Isr. J. Med. Sci.* **5**: 644–647.
33. Metropolitan Life Insurance Company Statistical Bulletin 40. November–December 1959.
34. Grundy, S. M., and A. L. Metzger. 1972. A physiological method for estimation of hepatic secretion of biliary lipids in man. *Gastroenterology.* **62**: 1200–1217.
35. Miettinen, T. A., E. H. Ahens, Jr., and S. M. Grundy. 1965. Quantitive isolation and gas-liquid chromatographic analysis of total dietary and fecal neutral steroids. *J. Lipid Res.* **6**: 411–424.
36. Grundy, S. M. 1975. Effects of polyunsaturated fats on lipid metabolism in patients with hypertriglyceridemia. *J. Clin. Invest.* **55**: 269–282.
37. Grundy, S. M., E. H. Ahrens, Jr., and T. A. Miettinen. 1965. Quantitative isolation and gas-liquid chromatographic analysis of total fecal bile acids. *J. Lipid Res.* **6**: 397–410.
38. Miettinen, T. A., E. H. Ahrens, Jr., and S. M. Grundy. 1965. Quantitative isolation and gas-liquid chromatographic analysis of total dietary and fecal neutral steroids. *J. Lipid Res.* **6**: 411–424.
39. Grundy, S. M., E. H. Ahrens, Jr., and G. Salen. 1968. Dietary β-sitosterol as an internal standard to correct for cholesterol losses in sterol balance studies. *J. Lipid Res.* **9**: 374–387.
40. Davignon, J., W. J. Simmonds, and E. H. Ahrens, Jr. 1968. Usefulness of chromic oxide as an internal standard for balance studies in formula-fed patients and for assessment of caloric function. *J. Clin. Invest.* **47**: 127–138.
41. Kessler, G., and H. Lederer. 1965. Fluorometric measurement of triglycerides. *In* Automation in Analytical Chemistry. Mediad Inc., New York. 341–344.
42. Thomas, P. J., and A. F. Hofmann. 1973. A simple calculation of the lithogenic index of bile: expressing biliary lipid composition on rectangular coordinates. *Gastroenterology.* **65**: 698–700.
43. Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods. Iowa State University Press, Ames, Iowa. 6th edition. 593.
44. Downie, N. M., and R. W. Heath. 1970. Basic statistical methods. Harper & Row, Publishers. New York. 3rd edition. 356.
45. Northfield, T. C., and A. F. Hofmann. 1973. Biliary lipid secretion in gallstone patients. *Lancet.* **1**: 747–748.
46. Redinger, R. N., and D. M. Small. 1972. Bile composition, bile salt metabolism, and gallstones. *Arch. Intern. Med.* **30**: 618–630.
47. Danzinger, R. G., H. Gordon, L. J. Schoenfield, and J. L. Thistle. 1972. Lithogenic bile in siblings of young women with cholelithiasis. *Mayo Clin. Proc.* **47**: 762–766.
48. Northfield, T. C., and A. F. Hofmann. 1973. Biliary lipid secretion in gallstone patients. *Lancet.* **1**: 747–748.
49. Garbutt, J. T., and T. J. Kenney. 1972. Effect of cholestyramine on bile acid metabolism in normal man. *J. Clin. Invest.* **15**: 2781–2789.
50. Kottke, B. A. 1969. Differences in bile acid excretion. Primary hypercholesteremia compared to combined hypercholesterolemia and hypertriglyceridemia. *Circulation.* **40**: 13–20.
51. Metzger, A. L., R. Adler, S. Heymsfield, and S. M. Grundy. 1973. Diurnal variation in biliary lipid composition. Possible role in cholesterol gallstone formation. *N. Engl. J. Med.* **288**: 333–336.
52. Edwards, P. A., H. Muroya, and R. G. Gould. 1972. In vivo demonstration of the circadian rhythm of cholesterol biosynthesis in the liver and intestine of the rat. *J. Lipid Res.* **13**: 396–401.
53. Shefer, S., S. Hauser, V. Lapar, and E. H. Mosbach. 1972. Diurnal variation of HMG CoA reductase activity in rat intestine. *J. Lipid Res.* **13**: 571–573.
54. Dietschy, J. M., and M. S. Brown. 1974. Effect of alterations of the specific activity of the intracellular acetyl CoA pool on apparent rates of hepatic cholesterogenesis. *J. Lipid Res.* **15**: 508–516.
55. Bagdade, J. D., E. L. Bierman, and D. Porte, Jr. 1967. The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects. *J. Clin. Invest.* **46**: 1549–1557.
56. Nepokroeff, C. M., M. R. Lakshmanan, G. C. Ness, R. E. Dugan, and J. W. Porter. 1974. Regulation of the diurnal rhythm of rat liver β-hydroxy-β-methylglutaryl coenzyme A reductase activity by insulin, glucagon, cyclic AMP, and hydrocortisone. *Arch. Biochem. Biophys.* **160**: 387–393.
57. Bhathena, S. J., J. Avigan, and M. E. Schreiner. 1974. Effect of insulin on sterol and fatty acid synthesis and hydroxymethylglutaryl CoA reductase activity in mammalian cells grown in culture. *Proc. Natl. Acad. Sci. U. S. A.* **71**: 2174–2178.
58. Schreibman, P. H. 1973. Cholesterol metabolism in human adipose tissue. *Clin. Res.* **21**: 638. (Abstr.)
59. Brunner, H., A. F. Hofmann, and W. H. J. Summerskill. 1972. Daily secretion of bile acids and cholesterol measured in health. *Gastroenterology.* **62**: 188. (Abstr.)
60. Schreibman, P., H. D. Pertsemlidis, G. C. K. Liu, and E. H. Ahrens, Jr. 1974. Lithogenic bile: a consequence of weight reduction. *J. Clin. Invest.* **53**: 72*a*. (Abstr.)